7.1 Bacterial consumption and transformation of dissolved organic matter (DOM) in the rivers Ob, Yenisei and the adjacent Kara-Sea.

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Introduction

Heterotrophic bacteria are the most abundant organisms in the oceans ($0.5 \times 10^6 - 10^7$ cells/ml) and constitute a key component in the oceanic organic carbon cycle (Azam et al. 1983; Williams 2000). Due to their almost unique ability to use dissolved organic matter (DOM) as carbon and energy source heterotrophic bacteria are the major sink of DOM thus shaping both the amount and composition of more than 95 % of the total organic carbon (TOC) in the oceans. While algal-derived organic carbon is predominantely labile and easily available to the bacterial community, riverine DOM is believed to be refractory due to the diagenetic processing the material has undergone on its way from the terrestrial source to the river mouth. Conservative mixing of riverine dissolved organic carbon (DOC) along the salt gradient from the freshwater to the marine endmembers supports the notion of a recalcitrant riverine DOC pool. However, the amount of terrestrially-derived carbon in the oceans and the influx of DOC through rivers are not ballanced i.e. significantly less terrestrially-derived carbon than expected can be measured in the marine environment (Hedges et al. 1997). Apparently there exists a major sink for terrestrial DOM that so far escaped recognition.

The Kara-Sea receiving the huge freshwater discharge of two of the largest rivers in the Arctic, Ob and Yenisei, is an ideal testground to investigate physicochemical DOM transformation processes during estuarine mixing and bacterial utilization of riverine DOM. A comprehensive overview of the sampling stations covered by the BP 2001 cruise is given in various other contributions of this volume.

In addition to taking water samples for routine DOC, dissolved organic nitrogen (DON) and inorganic nutrient monitoring, we measured an array of bacterial parameters at selected main stations covering the rivers Ob, Yenisei and the adjacent Kara-Sea. Furthermore, we performed a number of mixing experiments to address the impact of riverine DOM on bacterial growth in medium and high salinity water of the Kara-Sea Finally, we investigated the potential impact of photooxidation on riverine DOC concentration and transformation. Since information on the role of heterotrophic bacteria in the carbon cycle of the Arctic Ocean is still limited our study will be an important contribution to a better understanding of microbial processes and carbon fluxes in high latitude oceanic regimes.

Material and Methods

Dissolved organic matter (DOM) and inorganic nutrients

Water samples for the determination of DOC, fluorescent properties, dissolved amino acids and dissolved carbohydrates were filtered through precombusted GF/F-filters and stored in combusted ampoules (-20°C). GF/F-filtered samples for dissolved organic

nitrogen (DON; fixed with mercury chloride) and inorganic nutrient measurements were stored in the cold (4°C) or frozen (-20°C).

Ultrafiltration of DOM

In order to isolate DOM from the salty water matrix, prefiltered (0.2µm) large volume samples (150-500 l) were ultrafiltered using a tangential flow disc tube system (Pall Rochem). Filters (Desal, Osmonics) with cut-off sizes of about 150, 450, 800 and 2000 Daltons were applied, thus achieving concentrated samples for up to 4 different size classes of the DOM. All samples were concentrated to about 1.5 l and stored frozen at -20°C for detailed analyses. Analyses will include the elemental and isotopic composition of all size-fractions, allowing more detailed conclusions about the origin and diagenetic state of DOM.

In close cooperation with the Vernadsky Institute in Moscow analyses of ^{127}Cs and ^{90}Sr will be carried out in both the concentrated and permeated sample water. These results will improve our understanding about the interactions between DOM and artificial radionuclides.

Humic matter

For the isolation of humic matter filtered (0.2 µm) water samples (90 l) were acidified (HCl, pH 2) and pumped through a column filled with XAD-8 resin (Thurman and Malcolm, 1981). Hydrophobic substances (humic and fulvic acids) which are adsorbed by the resin under acidic conditions were eluted with an alkaline solution (0.1 M NaOH). Analyses on the humic matter extracts will include NMR-spectroscopy as well as the elemental and isotopic composition of humic and fulvic acids.

Lignin phenols

The DOM of pycnocline water samples previously collected during the BP 2000 cruise revealed elevated C/N ratios compared with surface and deep water samples. In order to gain more information on the source of the dissolved material in the pycnocline we concentrated DOM on C18 columns. During the BP 2001 cruise water from CTD-casts from the surface and the pycnocline of 15 selected stations was filtered (0.2 µm) and acidified with HCl to reach a pH of 2. Using a peristaltic pump we extracted 1 – 6 l of acidified sample water on C 18 columns (Varian Inc.) that had been prerinsed with 50 ml of MeOH and 1 l of deionized water (pH 2) just before sample processing. A subsequent rinse with 1 l of deionized water was applied to flush inorganic salts from the resin. The coulmns were stored frozen (-20°C). The absorbed material will be analyzed for lignin-phenols that are indicative for plant-derived material of terrestrial origin.

Bacterial numbers

Surface water samples (20 ml) from the Ob, Yenisei and depth profiles in the estuaries and the Kara Sea were fixed with 1 ml 0.2 µm-filtered formaldehyde (37 %) and stored in the cold. After a maximum of 2 days the fluorescent dye DAPI (2 µg/ml sample) was added to 2 - 5 ml of the fixed samples to stain the bacteria. After 3 minutes the stained bacteria were gently filtered on black polycarbonate filters. The filters were placed on a drop of immersion oil on a microscope slide. Finally the filters were covered by a cover slip on which a drop of oil had been smeared and frozen (-20°C) until counting with a epifluorescence microscope.

Bacterial production

Bacterial production measurements of water samples followed a modified procedure by Kirchman (1993). In short, ^{3}H-labeled leucine (final concentration: 10 nM) was added to 10 ml of a water sample. The samples were incubated for 1 to 2 h in the dark, generally at the in situ surface temperature of the respective station. After incubation the samples were filtered on 0.22 µm GSTF-filters (Millipore) using a Hoefer box. Immediately after filtration 2 ml of ice-cold 5 % TCA was added to the filters. After 2 minutes the TCA supernatant was discarded using vacuum filtration followed by a rinse with 2 ml of filtered, ice-cold seawater (or river water, depending on the salinity of the water sample). The filters were air dried and stored in scintillation vials. A sample set consisted of 3 replicates and a TCA-killed control with samples taken from the surface, the pycnocline and close to the bottom. In order to calcultate bacterial production from leucine uptake as accurate as possible we performed experiments to determine uptake/biomass conversion factors (Kirchman and Ducklow 1993) for the study area (Yenisei and Kara-Sea) rather than using non-site specific conversion factors from the literature. In total we performed about 770 individual assays during BP 2001 covering the depth profiles of 30 stations.

Bacterial uptake of sugars and amino acids

To estimate the contribution of sugars and amino acids to bacterial production we measured bacterial uptake of ^{3}H-glucose and a ^{3}H-labeled amino acid mixture in the rivers Ob, Yenisei and depth profiles of the adjacent Kara-Sea. The labeled compounds (final concentration: 0.5 nM) were added to 10 ml of sample water. The incubations lasted between 4 and 12 hours and were performed in the dark at the in situ surface temperature of the respective station. After the incubation the samples were filtered on 0.22 µm GSTF-filters (Millipore) followed by a rinse of the filters with 0.2 µm-filtered water taken at the sampling site. A sample set consisted of 2-3 replicates and one TCA-killed control. The filters were air dried and stored in scintillation vials. We performed 360 individual assays covering 17 stations in the study area.

Bacterial respiration

Bacterial respiration was measured in 0.8 µm-filtered water samples using high precision automated potentiometric Winkler determination of dissolved oxygen. Filtered water samples were allowed to sit for at least 30 minutes in PP-bottles before they were siphoned into 120 ml BOD-bottles carefully avoiding bubble formation. At least two bottle volumes of sample water were used to flush the BOD-bottles before the stoppers were added. One set of replicates (usually 4) was immediately fixed with Winkler reagents followed by the determination of dissolved oxygen in the titrator using 0.0125 N thiosulfate as titrant solution for 50 ml of sample aliquots. A second set of replicates was incubated for 22 to 50 h in the dark at the in situ water temperature before fixation and analysis of dissolved oxygen was performed. Differences in dissolved oxygen concentration between the start and the end of the experiments allow an estimate on bacterial oxygen consumption. Routinely, TCA-killed water samples were incubated along with untreated samples to check for abiotic oxygen consumption. The time consuming experimental design of the method allowed only a limited number of measurements. We measured bacteral respiration in surface and/or pycnocline water samples at 8 stations during the BP2001 cruise.

Carbon limitation of bacterial growth

We experimentally investigated possible carbon limitation of bacterial growth at 5 stations located in the rivers Yenisei, Ob and the Kara-Sea. Unfiltered surface water samples were filled into 2-l polycarbonate bottles. One bottle served as control and one bottle was spiked with glucose (2 µM final concentration) as carbon source. The bottles were incubated for about 72 hours in the dark at surface water temperature. In regular time intervals, subsamples were taken for bacterial production measurements using the method described above.

Impact of riverine water on bacterial growth in the Kara-Sea

In order to address the impact of riverine water on the growth of bacterial communities in the Kara Sea during estuarine mixing we performed 2 mixing experiments. We added 0.5 l of bacteria-free (0.2 µm-filtered) water of the Yenisei-endmember to polycarbonate bottles containing 1.5 l of freshly sampled surface water from the stations BP01-35 and BP01-66, respectively. The respective control bottles contained 1.5 liter of freshly sampled surface water and 0.5 l bacteria-free water from the same sampling site. Each experiment consisted of 2 replicates and one control. The bottles were incubated for 3-4 days in the dark at the surface water temperature. In regular intervals subsamples for bacterial production measurements and the determination of bacterial numbers and DOC concentrations were taken following the procedures described above.

Degradation of riverine DOM by bacteria

Raw water of the Yenisei, Ob and the Taz was stored in 20-50 l PP-tanks in the dark at approximately 4°C to study the degradation of riverine DOM by the natural bacterial communities and evaluate the amount of labile, semi-labile and refractory DOM present in the riverine water. Subsamples for DOC determination were taken in regular intervals during the cruise and sampling proceeded after arrival of the tanks at the Alfred-Wegener Institute in Bremerhaven.

Photooxidation of riverine DOM

Photooxidation by UV radiation is an important removal and transformation factor of DOM (Mopper and Kieber, 2000). We performed three experiments to adress the potential of photooxidation of riverine DOM in the Kara Sea. Filtered water (0.2 µm) of the Yenisei and Ob freshwater endmembers was exposed in quarz bottles to ambient sunlight on deck of the ship. The dissolved oxygen concentration in the sample water was determined at the beginning and at the end of the experiment using automated potentiometric Winkler determination (see above). In addition to oxygen measurements we took samples for DOC determinations in order to assess the formation of CO and CO_2 from dissolved organic carbon by photooxidation. The maximum exposure periods were 12 and 19 days, respectively, for water from the Yenisei and 2.5 days for Ob water.

Flocculation experiment

The abiotic conversion of DOM to particulate organic matter (POM) due to flocculation and sorption processes during estuarine mixing of riverine and marine water masses was investigated in a simple mixing experiment. Water from the Yenisei endmember and high salinity water from the Kara Sea were mixed in various proportions to give salinity values of 0, 2.5, 5.1, 6.8, 10.2 and 33.8 psu , respectively. We particularly focused on the salinity range between 0 and 10 psu because results from a previous experiment

indicated the predominance of flocculation processes in low-salinity mixtures. The mixed water samples were stored in 20 l plastic vessels in the dark at ambient temperature. After 2 days small volume subsamples were siphoned out of the vessels for DOC determination. The remaining water was filtered on precombusted GF/F filters for the determination of particulate organic carbon (POM). Samples for DOC and POM determinations of the endmembers were taken at the beginning of the experiment to calculate a theoretical mixing curve and assess deviations in the carbon partitioning caused by mixing.

Preliminary Results and Discussion

Bacterial respiration rates from 8 stations in the Kara Sea and the rivers Ob and Yenisei, respectively, are presented in Table 7.1. The measured rates range from below the limit of resolution (i.e. the difference in oxygen concentration between start and end of the incubations did not differ significantly when applying the student t-Test) to 2.2 μM O_2 d^{-1}, with most of the values around or below 1 μM and highest values encountered in the Ob bay (BP01-72) and north of the Ob bay (BP01-01). The few respiration data so far reported for the Arctic Ocean (Cota et al. 1996) give only numbers for community respiration on an area basis thus allowing no direct comparison to our study. A compilation of data on community respirations in various other oceanic regimes is presented by Williams (2000) with values generally ranging between 0.9 and 4.1 μM O_2 d^{-1}. Considering the fact that bacterial respiration generally represents only a fraction (about 40 %) of the community respiration our data from the Kara Sea aggree well with other oceanic regimes indicating an active bacterial community in arctic shelf areas. Interestingly, bacterial respiration in the pycnocline was generally higher than in the respective surface water sample suggesting favorable conditions for bacterial growth in the pycnocline, maybe due to the accumulation of particulate organic material in the steep density gradient. Bacterial production measurements that are currently processed in our laboratory, together with primary production measurements (E. Noethig and co-workers) will complete the data set and allow a more detailed and substantiated interpretation of the bacterial carbon demand and utilization in the Kara Sea.

The photoreactivity of the riverine DOM pool of the Yenisei is illustrated in Figure 7.1. Under sunny weather conditions we measured a photochemical dissolved oxygen consumption of 43 μM O_2 (3.6 μM O_2 d^{-1}) for Yenisei water within 12 days. The subsequent onset of cloudy conditions slightly decreased the rate to 2.9 μM O_2 d^{-1} with a total of 64 μM O_2 beeing consumed after 19 days of exposure to natural sunlight. A second experiment during cloudy conditions resulted in a decrease of 1.8 μM O_2 d^{-1} (Fig. 7.1) which is similar to a decrease of 1.5 μM O_2 d^{-1} measured in water of the Ob (data not shown). These oxygen consumption rates are more than an order of magnitude lower than reported values for the Amazon river system (3.6 μM O_2 d^{-1}; Amon and Benner 1996), reflecting the smaller photon flux in high latitudes. The magnitude of photooxidation processes in the estuaries of Ob and Yenisei is certainly restricted to the uppermost layer of the water column due to the strong light attenuation by the riverine humic substances. However, upon mixing with water of the Kara-Sea, the penetration of light increases and light quanta will reach photoreactive substances in deeper water layers. Integrated over the polar summer photooxidation in arctic waters might be an important factor for DOM transformation processes and the formation of labile bioreactive substrates from soil derived, recalcitrant carbon (Obernosterer et. al. 1999).

DOC concentrations of samples taken at the beginning and at the end of the experiments will reveal the potential for the direct formation of CO and CO_2 from DOC by photooxidation.

Table 7.1: Bacterial respiration in the Ob, Yenisei and the adjacent Kara Sea.

Station	Latitude °N	Longitude °E	Depth layer	Bacterial respiration ($\mu M\ O_2/d$)
BP01-01	74°59.12	76°23.41	surface	n.s.
			pycnocline	2.19
BP01-04	71°05.5	83°06.2	surface	n.s.
BP01-45	77°6.83	84°44.0	surface	0.62
			pycnocline	1.28
BP01-52	77°29.94	79°52.0	surface	0.41
			pycnocline	0.92
BP01-59	76°31.16	74°30.95	surface	n.s.
			pycnocline	0.34
BP01-65	75°42.98	75°50.79	surface	1.38
			pycnocline	0.77
BP01-68	74°35.05	72°14.97	surface	0.57
			pycnocline	1.01
BP01-72	70°49.88	73°44.34	surface	1.96

n.s. = no significant difference between start and end of incubation

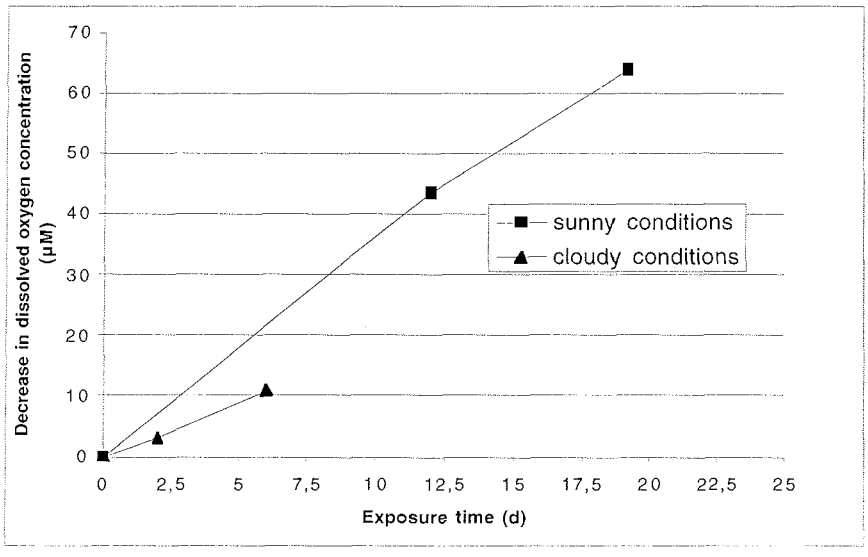

Figure.7.1: Photorespiration in 0.2 µm-filtered water from the Yenisei. Incubations were conducted in quarz bottles on deck during the BP 2001 cruise in August 2001 under natural light conditions.